OPEN ACCESS
Saudi Journal of Pathology and Microbiology
Abbreviated Key Title: Saudi J Pathol Microbiol
ISSN 2518-3362 (Print) |ISSN 2518-3370 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Saliva Based Nano-Biosensors for Early Detection of Oral Squamous Cell Carcinoma

RM Vatchalarani[1*], BC Manjunath[2], G Murli[3], Manas Bajpai[4], Mansi Mendiratta[5], Amit Kumar[6]

[1]Associate Professor, Department of Oral Pathology and Microbiology, Faculty of Dentistry, Jamia Millia Islamia, Jamianagar, New Delhi, India
[2]Senior Professor and Head, Department of Public Health Dentistry, Postgraduate Institute of Dental Sciences, Rohtak, Haryana, India
[3]Professor, Department of Prosthodontics, Faculty of Dentistry, Jamia Millia Islamia, Jamianagar, New Delhi, India
[4]Associate Professor, Department of Oral Pathology and Microbiology, NIMS Dental College, NIMS University, Jaipur, Rajasthan, India
[5]Junior Resident, Department of Public Health Dentistry, Postgraduate Institute of Dental Sciences, Rohtak, Haryana, India
[6]Junior Resident, Department of Public Health Dentistry, Postgraduate Institute of Dental Sciences, Rohtak, Haryana, India

DOI: 10.36348/sjpm.2021.v06i05.004 | **Received:** 09.04.2021 | **Accepted:** 15.05.2021 | **Published:** 18.05.2021

***Corresponding author:** RM Vatchala Rani

Abstract

Objectives: To provide an update regarding the recent trends and advancements in the field of salivary nano-biosensors which are useful in diagnosis of oral squamous cell carcinoma? ***Materials and methods*:** Literature survey was carried out in March 2021 in electronic databases such as PubMed SCOPUS, EMBASE, COCHRANRE library, Science Direct using key words such as Biosensors and oral cancer, Salivary biosensors and oral squamous cell carcinoma etc... Articles published in English language from 2000 to March 2021 which fulfilled the objectives of the study were included. ***Results*:** The searches revealed 236 articles out of which 42 were selected after reading the full text articles. Saliva based diagnostics is also referred as liquid biopsy for its immense potential as it is economical, noninvasive and easy to collect and contains wide variety of biomarkers which can be detected by biosensors. The use of biosensors in oral cancer detection and monitoring holds vast potential. Biosensors can be designed to detect emerging cancer biomarkers and to determine staging of the disease and diagnosis. Even though there are wide varieties of biomarkers, only few biosensors have the potential to become point of care diagnostics of oral cancer. ***Conclusions*:** The technological advancements in recent times have enabled researchers to develop high throughput and sensitive biosensors to detect active biomarkers from saliva of oral cancer patients.

Keywords: Salivary biosensor, salivary transcriptomes, salivary proteomics, salivary metabollomics, Cyfra 21.1.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is a major health problem in the world and particularly in countries like India. Oral cancers are the sixth most frequent cancer with a high mortality rate. It accounts for more than 90% of all oral cancers. The increased mortality and morbidity associated with oral cancer is due late diagnosis. The mortality can be decreased if oral cancer is detected at the earliest [1]. The gold standard for diagnosis is still tissue biopsy and histopathology, but various biomarkers are available which have good sensitivity and specificity that can diagnose (OSCC) at the early stage. Point of care diagnostics plays an important role in early diagnosis. As tissue biopsy takes longer time for diagnosis, point of care diagnostics will help in diagnosing oral squamous cell carcinoma in resource scarce settings [2, 3].

An instrument or a sensor which detects active biomarker of the disease is called a biosensor. They are devices used to detect the presence or concentration of a biological analyte, such as a biomolecule, a biological structure or a microorganism. Biosensors consist of three parts that is a component that recognizes the analyte and produces a signal, a signal transducer, and a reader device [4]. There are various types of biosensors such as enzyme based, tissue based, immunosensors, DNA biosensors, thermal and piezoelectric biosensors.

Human saliva is a valuable tool which can be used for diagnostics, as it contains numerous active metabolites that acts as biomarkers. The use of saliva for early cancer detection is a promising field and many investigators have reported regarding its utility. It is noninvasive, easy to collect and economical [5]. Saliva contains many active elements such as proteins, peptides, microbiomes, pro-inflammatory cytokines, monoclonal antibodies etc. secreted by major and minor salivary glands such as parotid gland, sublingual and sub-mental gland [6]. These active metabolites which are indicators of disease are often referred as biomakers. These are the molecular signatures which give inference regarding health and disease of an individual [7].

There is a direct contact between saliva and oral squamous cell carcinoma, because of which micro-molecular signatures are leached in to saliva from tumors. Salivary proteomics enhances diagnostics because of secretion of enzymes, cytokines, harmones, antibodies etc. from oral cancer cells. These signatures are taken up by various nano-biosensors which aid in diagnosis [8].

There are numerous salivary biomarkers based on genomics (DNA, RNA, mRNA), proteomics, transcriptomics, microbiomes, metballomics, pro-inflammatory cytokines (IL-6, IL-8, IL-1b, TNF-α) and others [5]. Many investigators have reported many biosensors which can detect active signatures of oral squamous cell carcinoma. Many biosensors have satisfactory sensitivity and specificity. Still it is under research and point of care diagnostics of oral squamous cell carcinoma is not freely available and hence, final diagnosis is based on tissue biopsy and histopathology [9-13]. Technological advancements in the nano-materials is taking place at a fast pace and in near future, point of care diagnostics based on nano-biosensors may be a reality.

However, further research is still required for the reliability and validation of salivary biomarkers and nano-biosensors for diagnostics of oral squamous cell carcinoma. Hence, the present review was carried with an objective to provide an update regarding the recent trends and advancements in the field of saliva based nano-biosensors which are useful in diagnosis of oral squamous cell carcinoma.

MATERIALS AND METHODS

Search strategy

A literature survey was carried out in various electronic data bases in March 2021 to identify the articles required for this narrative review on Nano-biosensors and oral cancer diagnosis. MeSH terms/keywords such as "biosensors and oral cancer" "biosensors and oral squamous cell carcinoma" "nanomaterials and oral cancer" "nanotechnology and oral cancer," were used to search in the electronic data bases such as PubMed data base, SCOPUS, EMBASE, COCHRANRE library, Science Direct and a manual search was also done using the cross references and textbooks. Articles published in English language from 2000 to March 2021 which fulfilled the objectives of the study were included.

Article selection criteria

The articles required for the review was selected based on the inclusion and exclusion criteria. Quality assessment was also carried out to select the articles required for this review.

Inclusion principles

1. Studies on characteristics and utility of biosensors in early detection of oral cancer
2. Studies on biosensors
3. Studies on biomarkers of oral cancer
4. Descriptive studies, Clinical trials, randomized controlled studies, Investigative reports.

Exclusion principles

1. Animal based studies
2. Narrative reviews on biosensors
3. Studies which did not use salivary nano-biosensors

RESULTS

The searches revealed 236 articles out of which 52 were selected after reading the titles and abstracts. After reading the full text articles and applying the inclusion and exclusion criteria 42 articles were selected for the review which fulfilled the objectives of the study.

Table-1: Salivary biomarkers of oral squamous cell carcinoma

Study	Type of biomarker	Biomarker	Nanotechnology
Jafari M and, Mohammad M 2020 [14]	antigen and antibody immune-complex	Cys-GA-anti-Cyfra21.1-BSA-Cyfra21.1	Au electrode was modified by Cysteamine (CysA) and Glutaraldehyde (GA) respectively via self-assembly as a substrate to immobilize the biological agents.
Song CK *et al*. 2018 [15]	Cytokeratin-19 antigen	Cyfra 21-1	hierarchical three-dimensional network of carbon nanotubes on Si pillar substrate (3DN-CNTs)
Lee LT *et al*. 2018 [16]	Pro inflammatory cytokines	IL-1β, IL-6, IL-8, MIP-1β, eotaxin and IFN-γ and TNF-α	Luminex Bead-based Multiplex Assay.
St John MA *et al*. 2004 [17]	Pro inflammatory cytokines	IL-6 and IL-8	quantitative real-time polymerase chain reaction analysis and enzyme-linked immunosorbent assay,
Nakamichi E *et al*. 2021 [18]	Salivary exosomal Alix	exoAlix	Enzyme-linked immunosorbent assay.(ELISA)
Piesker A *et al*. 2017 [19]	salivary metalloproteinase-9	MMP-9	Enzyme-linked immunosorbent assay (ELISA)
He L *et al*. 2020 [20]	miRNAs in salivary exosomes	overexpression of miR-24-3p	miRNA microarray analysis, then verified by qRT-PCR.
Gai C *et al*. 2018 [21]	micro RNAs (miRNAs	miR-512-3p and miR-412-3p were up-regulated in salivary EVs	qRT-PCR array and qRT-PCR.
Ueda S *et al*. [22]	salivary microbiome.	significant increase in the expression level of CCL20	microarray and qRT-PCR
Li Y *et al*. 2004 [23]	salivary transcriptomes	Potential salivary RNA biomarkers are transcripts of IL8, IL1B, DUSP1, HA3, OAZ1, S100P, and SAT.	microarray and qRT-PCR
Martin JW *et al*. 2015 [24]	salivary transcriptomes	mRNAs (IL1β, IL8, OAZ1, SAT, S100P, and DUSP1)	microarray and qRT-PCR
Aziz S *et al*. 2015 [25]	Immunosuppressive cytokines	IL-4, IL-10, IL-13, and IL-1RA were elevated	microarray and qRT-PCR
Kamatani T *et al*. 2013 [26]	Proinflammatory cytokines	IL-1 beta in UWS may be useful for detection of early stage OSCC.	Bio-Plex suspension array system.
Zhong LP 2007 [27]	soluble fragment of cytokeratin 19(CK19)	Saliva Cyfra 21-1 concentration in OSCC patients was significantly higher	immunohistochemistry and fluorescent real-time RT-PCR
Awashthi N *et al*. 2017	cytokeratin 19(CK19)	salivary CYFRA 21-1, LDH, total protein, and amylase	immunohistochemistry and fluorescent real-time RT-PCR
Rajkumar K *et al*. 2015 [28]	intermediate filament proteins of epithelial cells	significant increase in CYFRA 21-1 level in OSCC	Immunohistochemistry and fluorescent real-time RT-PCR
Conolly JM *et al*. 2016 [30]	SERS spectra from oral cells	oral cells surface-enhanced Raman spectroscopy (SERS)	silver nanoparticle-based surface-enhanced Raman spectroscopy (SERS) as a label-free, non-invasive technique
Kumar S *et al*. 2015 [12]	Monoclonal antibodies	anti-CYFRA-21-1	Silanized nanostructured zirconia, electrophoretically deposited onto indium tin oxide (ITO) coated glass for covalent immobilization of the monoclonal antibodies (anti-CYFRA-21-1).
Zhang Y *et al*. 2015 [31]	Proinflammatory cytokines	IL-8 and TNF-α detection in saliva	Silicon nanowire (SiNW) field-effect transistor (FET) biosensors
Song X *et al*. 2020 [32]	wide range of metabolite species	dysregulated metabolites	Conductive polymer spray ionization mass spectrometry (CPSI-MS) and desorption electrospray ionization MS imaging (DESI-MSI),

Biosensors for diagnosis of oral squamous cell carcinoma

Song X *et al*. 2020 [32] reported regarding metabolite signatures in human saliva of patients suffering from oral squamous cell carcinoma. Investigators employed conductive polymer spray ionization mass spectrometry (CPSI-MS) to record a wide range of metabolite species within a few seconds, making this technique appealing as a point-of-care method for the early detection of OSCC. They discovered and validated dysregulated metabolites and altered metabolic pathways.

Kumar S *et al*. 2021[11] reported regarding the fabrication of yttria-doped zirconia-reduced graphene oxide nanocomposite (nYZR) based biosensing platform for detection of salivary CYFRA-21-1 biomarker. This work opens new opportunities to explore the electrochemical behavior of nanostructured yttria stabilized zirconia (YSZ) and its composites at room temperature and its utility in developing biosensors and other electrochemical devices.

Kumar S *et al*. 2015 [12] reported regarding application of the silanized nanostructured zirconia, electrophoretically deposited onto indium tin oxide (ITO) coated glass for covalent immobilization of the monoclonal antibodies (anti-CYFRA-21-1). This biosensing platform has been utilized for a simple, efficient, noninvasive, and label-free detection of oral cancer via cyclic voltammetry technique. The results of these studies have been validated via enzyme-linked immunosorbent assay.

Jafari M *et al*. 2020 [14] reported regarding an efficient immunosensor (Cys-GA-anti-Cyfra21.1-BSA-Cyfra21.1 antigen/AuE) which was successfully designed and developed for the detection and determination of the Cyfra21.1 biomarker in unprocessed human saliva samples. The engineered immunosensor exhibited an excellent ability to detect and determine of Cyfra21.1 biomarker in low concentrations in unprocessed human saliva samples. Based on results, this biosensor can provide appropriate, reliable, affordable, quick, and user-friendly diagnostic device to monitoring oral abnormality by detection and determination of Cyfra21.1 biomarker in human saliva.

Song CK *et al*. 2018 [15] reported regarding a hierarchical three-dimensional network of carbon nanotubes on Si pillar substrate (3DN-CNTs) which was developed for the accurate detection of oral squamous cell carcinoma (OSCC) in clinical saliva samples. Cytokeratin-19 antigen (Cyfra 21-1) was utilized as a model biomarker of OSCC for fluorescence-based immunosensor using 3DN-CNTs (3DN-CNTs sensor). The 3DN-CNTs sensor enhances the sensitivity of Cyfra 21-1 detection by increasing the density of immobilized antibody through high surface area of 3DN-CNTs and enhancing the accessibility of biomolecules through the ordered pathway of hierarchical structure.

DISCUSSION

Biosensors are useful diagnostic tool for early detection of cancer. A wide range of biosensors for cancer diagnostics include aptamers, enzymes, DNA probes, fluorescent probes, interacting proteins and antibodies in vicinity to transducers such as electrochemical, optical and piezoelectric [33]. Efforts are being made to develop point of care biosensor for detecting oral squamous cell carcinoma.

Oral cancer is the one of most common cancer in the world. The gold standard for diagnosis is still tissue biopsy, cytology and histopathology. Saliva based diagnostics is also referred as liquid biopsy for its immense potential as it is economical, noninvasive, easy to collect and contains wide variety of biomarkers which can be detected by biosensors. The use of biosensors in oral cancer detection and monitoring holds vast potential. Biosensors can be designed to detect emerging cancer biomarkers and to determine staging of the disease and diagnosis. Even though there are wide varieties of biomarkers, only few biosensors have the potential to become point of care diagnostics of oral cancer [10-12].

Many investigators have focused on Salivary Cyfra 21-1 concentration in OSCC patients which was significantly higher. In malignant tissues, the intermediate filament known as cytokeratin 19 and the C-terminus of cytokeratin 19 (CYFRA 21-1, CYFRA) are released into circulation by a cleaving enzyme, caspase-3, and apoptosis. These signatures are taken up by various biosensors [14].

Kumar S *et al*. [11], Jafari M *et al*. [14], song CK *et al*.[15], Kumar S *et al*. [12] have reported biosensors based on various nano-materials to detect Cyfra 21.1. It has been reported that these biosensors have high sensitivity and low specificity when compared to tissue biopsies but has its own disadvantages. Salivary Cyfra 21.1 is also a biomarker for various other cancers like lung cancer [34]. Most of the biosensors have low specificity that is ability to detect true negative cases.

Recent advancements in technologies, such as mass spectrometry, liquid chromatography, and protein/peptide labeling technologies have made it possible for detection of proteomes in human saliva at low concentrations [35]. Numerous studies have reported that the proteomic profile of saliva from OSCC patients differs from the profile for OSCC-free controls (Table 1). The findings of study by Peisker *et al*. [19]

have revealed that salivary metalloproteinase-9 was found to be in high abundance in patients with OSCC.

Salivary transcriptome also plays a role as an important biomarker and extracellular RNA is an emerging diagnostic technology due to its discriminatory power for disease detection. High-throughput microarray technology has made the investigation of gene expression on a genome-wide level feasible and routine [36]. Li Y *et al*. 2004 [23] have reported that potential RNA biomarkers such as transcripts of IL8, IL1B, DUSP1, HA3, OAZ1, S100P, and SAT can be detected in human saliva of OSCC patients. In a research study conducted by Martin JW *et al*. [24], it has been revealed that saliva of OSCC patients contained abundant quantities of mRNAs (IL1β, IL8, OAZ1, SAT, S100P, and DUSP1 when compared to controls (No OSCC). The findings of the study by Gai C *et al*. [21] has revealed that miR-512-3p and miR-412-3p were up-regulated in salivary EVs of OSCC patients when compared to the controls giving an indication that salivary transcriptomes can be used as an important biomarker for early detection of OSCC.

Metabolomics is a measure of all intracellular metabolites and is a potent tool for understanding cellular function [36]. Metabolomics-based technology is emerging for the identification of disease-associated salivary analytes. Song X *et al*. [32] have found out a wide range of metabolite species which were dysregulated metabolites found in abundance in OSCC patients. They used conductive polymer spray ionization mass spectrometry (CPSI-MS) and desorption electrospray ionization MS imaging (DESI-MSI) to detect the active metabolites in the saliva of OSCC patients.

Researchers worldwide are focusing on salivary diagnostics of oral squamous cell carcinoma because of availability of potential biomarkers which can be used oral cancer diagnostics. There are certain drawbacks as high throughput and sensitive technology is essential to detect biomarkers in small quantities to arrive at a diagnosis [37]. Many researchers are now investigating to find out an electrochemical biosensor capable of identifying salivary biomarkers with high sensitivity and specificity.

CONCLUSIONS

The technological advancements in recent times have enabled researchers to develop high throughput and sensitive biosensors to detect active biomarkers from saliva of oral cancer patients. The field is still in nascent stage and there is tremendous amount of scope in future to develop point of care biosensors for early detection of oral squamous cell carcinoma using human saliva which is easy to collect and economical.

REFERENCES

1. D'souza, S., Addepalli, V. (2018). Preventive measures in oral cancer: An overview. Biomed Pharmacother, 107, 72-80.
2. Bossmann, S.H., Troyer, D.L. (2013). Point-of-care routine rapid screening: the future of cancer diagnosis? Expert Rev Mol Diagn, 13(2), 107-9.
3. Aro, K., Kaczor-Urbanowicz, K., Carreras-Presas, C.M. (2019). Salivaomics in oral cancer Curr Opin Otolaryngol Head Neck Surg, 27(2), 91-97.
4. Mehrotra, P. (2016). Biosensors and their applications - A review. J Oral Biol Craniofac Res, 6(2), 153-9.
5. Malon, R.S., Sadir, S., Balakrishnan, M., Córcoles, E.P. (2014). Saliva-based biosensors: noninvasive monitoring tool for clinical diagnostics. Biomed Res Int, 962903. doi: 10.1155/2014/962903.
6. Chattopadhyay, I., Verma, M., Panda, M. (2019). Role of Oral Microbiome Signatures in Diagnosis and Prognosis of Oral Cancer. Technol Cancer Res Treat, 1(18), 153-60.
7. Khurshid, Z., Zafar, M.S., Khan, R.S., Najeeb, S., Slowey, P.D., Rehman, I.U. (2018). Role of Salivary Biomarkers in Oral Cancer Detection. Adv Clin Chem, 86, 23-70.
8. Li, Q., Ouyang, X., Chen, J., Zhang, P., Feng, Y. (2020). A Review on Salivary Proteomics for Oral Cancer Screening. Curr Issues Mol Biol, 37, 47-56.
9. Rapado-González, Ó., Martínez-Reglero, C., Salgado-Barreira, Á., Takkouche, B., López-López, R., Suárez-Cunqueiro, M.M., Muinelo-Romay, L. (2020). Salivary biomarkers for cancer diagnosis: a meta-analysis. Ann Med, 52(3-4), 131-144.
10. Kumar, S., Sharma, J.G., Maji, S., Malhotra, B.D. (2016). Nanostructured zirconia decorated reduced graphene oxide based efficient biosensing platform for non-invasive oral cancer detection. Biosens Bioelectron, 15(74), 97-504
11. Kumar, S., Gupta, N., Malhotra, B.D. (2021). Ultrasensitive biosensing platform based on yttria doped zirconia-reduced graphene oxide nanocomposite for detection of salivary oral cancer biomarker. Bioelectrochemistry, 140, 107799.
12. Kumar, S., Kumar, S., Tiwari, S., Srivastava, S., Srivastava, M., Yadav, B.K., Kumar, S., Tran, T.T., Dewan, A.K., Mulchandani, A., Sharma, J.G., Maji, S., Malhotra, B.D. (2015). Biofunctionalized Nanostructured Zirconia for Biomedical Application: A Smart Approach for Oral Cancer Detection. Adv Sci (Weinh), 2(8), 1500048.
13. Zhang, Y., Chen, R., Xu, L., Ning, Y., Xie, S., Zhang, G.J. (2015). Silicon nanowire biosensor for highly sensitive and multiplexed detection of oral squamous cell carcinoma biomarkers in saliva. Anal Sci, 31(2), 73-8.
14. Jafari, M., Hasanzadeh, M. (2020). Non-invasive bioassay of Cytokeratin Fragment 21.1 (Cyfra 21.1) protein in human saliva samples using immunoreaction method: An efficient platform for early-stage diagnosis of oral cancer based on biomedicine Biomed Pharmacother, 131, and 110671.
15. Song, C.K., Oh, E., Kang, M.S., Shin, B.S., Han, S.Y., Jung, M., Lee, E.S., Yoon, S.Y., Sung, M.M.,

Ng, W.B., Cho, N.J., Lee, H. (2018). Fluorescence-based immunosensor using three-dimensional CNT network structure for sensitive and reproducible detection of oral squamous cell carcinoma biomarker Anal Chim Acta, 16(1027),101-108.

16. Lee, L.T., Wong, Y.K., Hsiao, H.Y., Wang, Y.W., Chan, M.Y., Chang, K.W. (2018). Evaluation of saliva and plasma cytokine biomarkers in patients with oral squamous cell carcinoma. Int J Oral Maxillofac Surg, 47(6), 699-707.
17. St John, M.A., Li, Y., Zhou, X., Denny, P., Ho, C.M., Montemagno, C., Shi, W., Qi, F., Wu, B., Sinha, U., Jordan, R., Wolinsky, L., Park, N.H., Liu, H., Abemayor, E., Wong D.T. (2004). Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. Arch Otolaryngol Head Neck Surg. 130(8), 929-35.
18. Nakamichi, E., Sakakura, H., Mii, S., Yamamoto, N., Hibi, H., Asai, M., Takahashi, M. (2021). Detection of serum/salivary exosomal Alix in patients with oral squamous cell carcinoma. Oral Dis, 27(3), 439-447.
19. Peisker, A., Raschke, G.F., Fahmy, M.D., Guentsch, A., Roshanghias, K., Hennings, J., Schultze-Mosgau, S. (2017). Salivary MMP-9 in the detection of oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal, 22(3), e270-275.
20. He, L., Ping, F., Fan, Z., Zhang, C., Deng, M., Cheng, B., Xia, J. (2020). Salivary exosomal miR-24-3p serves as a potential detective biomarker for oral squamous cell carcinoma screening. Biomed Pharmacother, 121, 109553.
21. Gai, C., Camussi, F., Broccoletti, R., Gambino, A., Cabras, M., Molinaro, L., Carossa, S., Camussi, G., Arduino, P.G. (2018). Salivary extracellular vesicle-associated miRNAs as potential biomarkers in oral squamous cell carcinoma. BMC Cancer, 18(1), 439.
22. Ueda, S., Goto, M., Hashimoto, K., Hasegawa, S., Imazawa, M., Takahashi, M., Oh-Iwa I., Shimozato, K., Nagao, T., Nomoto, S. (2021). Salivary CCL20 Level as a Biomarker for Oral Squamous Cell Carcinoma. Cancer Genomics Proteomics, 18(2), 103-112.
23. Li, Y., St John, M.A., Zhou, X., Kim, Y., Sinha, U., Jordan, R.C., Eisele, D., Abemayor E., Elashoff, D., Park, N.H., Wong, D.T. (2004). Salivary transcriptome diagnostics for oral cancer detection. Clin Cancer Res, 10(24), 8442-50.
24. Martin, J.L., Gottehrer, N., Zalesin, H., Hoff, P.T., Shaw, M., Clarkson, J.H., Haan, P., Vartanian, M., McLeod, T., Swanick, S.M. (2015). Evaluation of Salivary Transcriptome Markers for the Early Detection of Oral Squamous Cell Cancer in a Prospective Blinded Trial. Compend Contin Educ Dent, 36(5), 365-73.
25. Aziz, S., Ahmed, S.S., Ali, A., Khan, F.A., Zulfiqar, G., Iqbal, J., Khan, A.A., Shoaib, M. (2015). Salivary Immunosuppressive Cytokines IL-10 and IL-13 Are Significantly Elevated in Oral Squamous Cell Carcinoma Patients. Cancer Invest, 33(7), 318-28.
26. Kamatani, T., Shiogama, S., Yoshihama, Y., Kondo, S., Shirota, T., Shintani, S. (2013). Interleukin-1 beta in unstimulated whole saliva is a potential biomarker for oral squamous cell carcinoma. Cytokine, 64(2), 497-502.
27. Zhong, L.P., Zhang, C.P., Zheng, J.W., Li, J., Chen, W.T., Zhang, Z.Y. (2007). Increased Cyfra 21-1 concentration in saliva from primary oral squamous cell carcinoma patients. Arch Oral Biol, 52(11), 1079-87.
28. Awasthi, N. (2017). Role of salivary biomarkers in early detection of oral squamous cell carcinoma. Indian J Pathol Microbiol, 60(4), 464-468.
29. Rajkumar, K., Ramya, R., Nandhini, G., Rajashree, P., Ramesh, Kumar, A., Nirmala Anandan, S. (2015). Salivary and serum level of CYFRA 21-1 in oral precancer and oral squamous cell carcinoma. Oral Dis, 21(1), 90-6.
30. Connolly, J.M., Davies, K., Kazakeviciute, A., Wheatley, A.M., Dockery, P., Keogh, I., Olivo, M. (2016). Non-invasive and label-free detection of oral squamous cell carcinoma using saliva surface-enhanced Raman spectroscopy and multivariate analysis. Nanomedicine, 12(6), 1593-601.
31. Zhang, Y., Chen, R., Xu, L., Ning, Y., Xie, S., Zhang, G.J. (2015). Silicon nanowire biosensor for highly sensitive and multiplexed detection of oral squamous cell carcinoma biomarkers in saliva. Anal Sci, 31(2), 73-8.
32. Song, X., Yang, X., Narayanan, R., Shankar, V., Ethiraj, S., Wang, X., Duan, N., Ni, Y.H., Hu, Q., Zare, R.N. (2020). Oral squamous cell carcinoma diagnosed from saliva metabolic profiling. Proc Natl Acad Sci U S A, 117(28), 16167-16173.
33. Ranjan, R., Esimbekova, E.N., Kratasyuk, V.A. (2017). Rapid biosensing tools for cancer biomarkers. Biosens Bioelectron, 87, 918-930.
34. Kammer, M.N., Kussrow, A.K., Webster, R.L., Chen, H., Hoeksema, M., Christenson, R., Massion, P.P., Bornhop, D.J. (2019). Compensated Interferometry Measures of CYFRA 21-1 Improve Diagnosis of Lung Cancer. ACS Comb Sci, 21(6), 465-472.
35. Chu, H.W., Chang, K.P., Hsu, C.W., Chang, I.Y., Liu, H.P., Chen, Y.T., Wu, C.C. (2019). Identification of Salivary Biomarkers for Oral Cancer Detection with Untargeted and Targeted Quantitative Proteomics Approaches. Mol Cell Proteomics, 18(9), 1796-1806.
36. Santosh, A.B., Jones, T., Harvey, J. (2016). A review on oral cancer biomarkers: Understanding the past and learning from the present. J Cancer Res Ther, 12(2), 486-92.
37. Kaur, J., Jacobs, R., Huang, Y., Salvo, N., Politis, C. (2018). Salivary biomarkers for oral cancer and pre-cancer screening: a review. Clin Oral Investig, 22(2), 633-640.